Active Ingredients in Cade Oil That Synergize Attractiveness of α-Ionol to Male *Bactrocera latifrons* (Diptera: Tephritidae)

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ABSTRACT Cade oil, a commercially available essential oil produced by destructive distillation of juniper, *Juniperus oxycedrus* L., twigs, is known to synergize the attractancy of α-ionol to male *Bactrocera latifrons* (Hendel). Through chemical fractionation and outdoor olfactometer-based bioassays, seven compounds in cade oil were identified that potentially could provide some level of synergism. Tests with sterile laboratory flies showed that four of the seven compounds (eugenol, isoeugenol, 2-methoxy-4-ethylphenol, and 2-methoxy-4-propylphenol), together with a closely related compound not found in cade oil, 2-methoxy-4-methylphenol, are capable of synergizing the attractiveness of α-ionol to male *B. latifrons* under field conditions. The similarity in structures of these five synergistic compounds shows that there is a response to a core 2-methoxyphenol structure, with fly response little affected by some variation in the composition of the side chain on the number 4 carbon. Because identified synergists were structurally similar, only one compound, eugenol, was selected for further field studies. In an 8-wk weathering test, using released sterile flies, traps baited with α-ionol + eugenol had catches comparable with catches at traps baited with α-ionol + cade oil, with catches generally increased with a higher eugenol loading. For both eugenol and cade oil, catches tended to be better when these synergists were deployed on separate wicks from the α-ionol. Eugenol and α-ionol, however, were unable to provide attraction comparable with that of cade oil and α-ionol in tests with wild fly populations.

KEY WORDS *Bactrocera latifrons*, cade oil, α-ionol, eugenol

THE SOLANACEOUS FRUIT FLY, *Bactrocera latifrons* (Hendel), is a tephritid fruit fly that primarily infests solanaceous fruits but has also been found to infest some cucurbitaceous fruits (Liquido et al. 1994). Since its discovery in Hawaii in 1983 (Vargas and Nishida 1985), it has spread throughout the state of Hawaii (Liquido et al. 1994). Although, at present, little economic damage has been attributed to this species, it has the potential to impact production of cucurbitaceous and especially solanaceous crops such as peppers, *Capsicum annuum* L. and *Capsicum frutescens* L. The development of a male attractant for *B. latifrons* began with the discovery of α-ionol (Flath et al. 1994), now commonly referred to as latilure (McGovern et al. 1989). The attractiveness of this lure was subsequently found to be synergistically enhanced by cade oil (Liquido et al. 2000, McQuate and Peck 2001). Cade oil is a commercially available essential oil produced by destructive distillation of juniper, *Juniperus oxycedrus* L., twigs. Because cade oil is a multicompound distillation product, it was not known which chemical(s) in cade oil was responsible for the synergistic effect. In the course of seeking to identify the active ingredients, >200 compounds were identified within cade oil. Through a process of preparation of chemical fractions of cade oil and subsequent bioassays in an outdoor olfactometer with sexually mature solanaceous fruit flies, seven compounds were selected for further field testing. These compounds, whose structural formulas are shown in Fig. 1, have the following approximate concentrations in cade oil: crotonaldehyde (0.63%), eugenol (0.087%), *o*-eugenol (0.25%), isoeugenol (0.073%), isovanillin (0.043%), 2-methoxy-4-ethylphenol (0.20%), and 2-methoxy-4-propylphenol (0.12%) (Y.-S.K., unpublished data). In a preliminary field test involving low concentrations of the compounds, traps baited with four of these compounds, each presented together with α-ionol, showed significantly higher catch of male solanaceous fruit flies than traps baited with α-ionol alone, but significantly lower catch than traps baited with α-ionol and cade oil (unpublished data). The methodology used in isolating the potential active ingredients in cade oil and the initial results of primarily olfactometer-based

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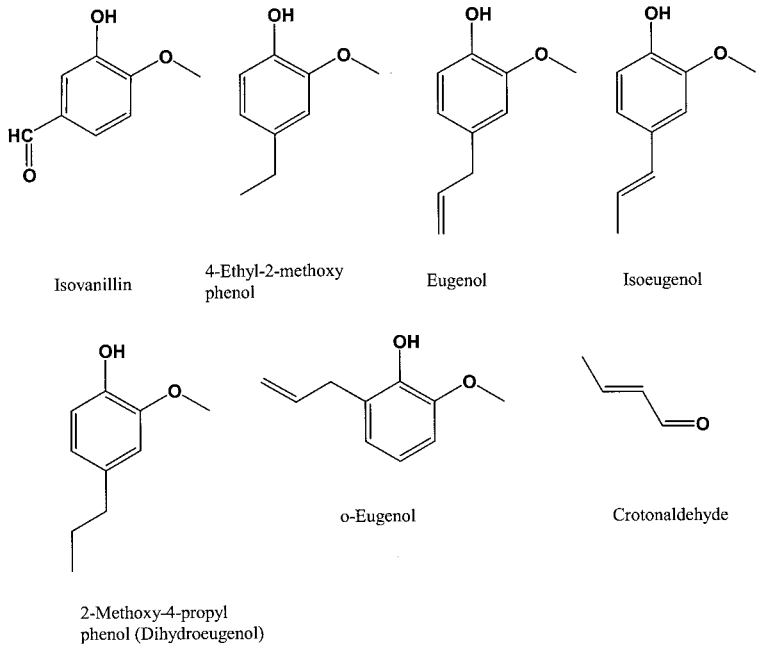


Fig. 1. Structural formulas for the seven cade oil constituents identified through preparation of chemical fractions followed by outdoor olfactometer-based bioassays as potentially synergistic with α-ionol in attracting male *B. latifrons*.

bioassays, whereas, in this article, we report on field trials with both sterile and wild flies to assess the effectiveness of these identified compounds as α-ionol synergists.

Materials and Methods

Chemicals. α-Ionol, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-ol, was obtained from Bedoukian Research, Inc. (Danbury, CT). Rectified cade oil was obtained from Penta Manufacturing (West Caldwell, NJ). 2-Methoxy-4-ethylphenol and 2-methoxy-4-methylphenol were obtained from TCI America (Portland, OR). Crotonaldehyde, eugenol, *o*-eugenol, isoeugenol, isovanillin, and 2-methoxy-4-propylphenol were all obtained from Sigma (St. Louis, MO).

Insects. Sterile *B. latifrons* pupae used to provide adult flies for olfactometer and field tests were obtained from a laboratory colony at the USDA–ARS U.S. Pacific Basin Agricultural Research Center in Honolulu, HI. Fruit flies used in our tests were kept in an insectary at 24–27°C, 65–70% RH, and a photoperiod of 12:12 (L:D) h. Adults were fed water and a diet of three parts sucrose, one part protein yeast hydrolysate (Enzymatic, United States Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhinelander Paper Co., Rhinelander, WI). Fifteen- to 20-d-old sexually mature flies were used in the field studies.

Field Tests with Sterile Laboratory Flies. *Study 1. Initial Field Trials.* Concentrations of the seven identified compounds in cade oil ranged from 430 to 6,300 ppm (unpublished data). From within this range, a higher dose (5,000 ppm) was selected for initial testing because there could be increased response at a higher dose and, also, because it could provide more prolonged release for highly volatile compounds, such as crotonaldehyde. The 5,000 ppm solution of each of the seven identified compounds was prepared in methanol. Field trials were conducted using two different volume ratios (1:1 and 4:1) of potential synergist solution to α-ionol to see whether a higher concentration of synergist could improve its performance. Results of a 1:4 ratio will be reported elsewhere. In test A, Jackson traps held cotton wicks treated with 0.4 ml of synergist solution and 0.4 ml of α-ionol on separate wicks, giving an effective ratio of 1:200. In test B, Jackson traps held wicks treated with 1.6 ml of synergist solution and 0.4 ml of α-ionol on separate wicks, giving an effective ratio of 1:50. Each of these tests also included treatments with 0.1 ml of cade oil and 0.4 ml of α-ionol (on separate wicks), with 0.4 ml of α-ionol alone, and with 0.4 ml of water alone. For each test, five traps of each treatment were set out in a random complete block design in a macadamia nut orchard (a nonhost environment). Traps were placed in every tree (one trap per tree) down a row (4.6-m spacing) with replicate blocks in adjacent rows (9.2 m spacing). Approximately 4,500 sexually mature *B. latifrons* adults were uniformly released from holding containers in the aisles between tree rows and beyond the outer tree rows with traps. Traps were retrieved 24 h after the fly release. Tests A and B were all repeated so that there were a total of three replications for each test.

Study 2. Trials with Pure Compounds. The four compounds in study 1 with the best synergistic effect (eugenol, isoeugenol, 2-methoxy-4-ethylphenol, and 2-methoxy-4-propylphenol) were selected for a further trial by using pure compound (i.e., not prepared in methanol). Use of pure compound made it easier to test higher loadings of the synergist, which seemed justified, because higher loadings with the 5,000 ppm preparations led to improved response relative to the cade oil response. Because all of these compounds were similar structurally, we chose to include another structurally similar compound (but one that had not been found in cade oil), 2-methoxy-4-methyl phenol, to test for synergistic effect. Because multiple synergizing compounds were identified in cade oil, we also looked at the combinations eugenol and isoeugenol and 2-methoxy-4-ethylphenol and 2-methoxy-4-propylphenol. In these tests, 0.1 ml of the test compound and 1.0 ml of α-ionol were presented on separate wicks as described for study 1, except for the double synergist treatments. In these treatments, 0.05 ml of each compound was added separately to opposite ends of the same wick. We also included 0.1 ml of cade oil + 1.0 ml of α-ionol, 1.0 ml of α-ionol, and 1.0 ml of water treatments. Tests were run with five traps of each treatment set out in a random complete block design in a macadamia nut orchard, as described for study 1. A total of three replicates of this trial were completed.

Study 3. Weathering Test with Eugenol. Because study 2 showed little difference in synergistic effect among test compounds, and because compounds were structurally similar, we selected only one of the best-performing compounds for weathering tests. Based on perceived relative safety of use and lower cost, eugenol was selected. We tested two eugenol:α-ionol ratios, 1:10 and 1:2. Treatments included 0.2 ml of eugenol + 2.0 ml of α-ionol, 1.0 ml of eugenol + 2.0 ml of α-ionol, 0.2 ml of cade oil + 2.0 ml of α-ionol, 1.0 ml of eugenol alone, 2.0 ml of α-ionol alone, and 2.0 ml of water alone. With the exception of the water treatment, all treatments were presented both with wicks freshly treated immediately before the fly release for each test week and with wicks allowed to age over the course of the test. Traps were set out in a macadamia nut orchard in a randomized complete block design as described for studies 1 and 2. Sexually mature sterile *B. latifrons* adults were released uniformly throughout the trapping grid on weeks 0, 1, 2, 4, 6, and 8, with sticky inserts recovered 24 h after each fly release.

Study 4. Test of Mixing Effect. α-Ionol + cade oil has been effectively used both when presented on separate wicks and as a mixture added to a single wick, but the effect of mixing eugenol with α-ionol had not been tested. We compared catch response with mixed versus unmixed eugenol (1.0 ml) + α-ionol (2.0 ml) as well as mixed versus unmixed cade oil (1.0 ml) + α-ionol (2.0 ml). Both mixed and unmixed treatments were presented both freshly treated before fly release (fresh wicks) and allowed to age over the course of the test (aged wicks). Treatments with 2.0 ml of α-ionol alone, both fresh and aged, were also included as was a 2.0 ml of water treatment. Traps were set out in a macadamia nut orchard in a randomized complete block design as described for studies 1 and 2. Sexually mature sterile *B. latifrons* adults were released uniformly throughout the trapping grid on day 0 and weeks 2, 4, and 6 with sticky inserts recovered 24 h after each fly release.

Study 5. Documentation of Synergistic Effect and Oriental Fruit Fly Response. Although previous research (McQuate and Peck 2001) showed that cade oil synergistically enhanced the attractiveness of α-ionol to male *B. latifrons*, we wanted to document that the identified active ingredients also showed a similar synergistic effect. Additionally, because of the similarity of eugenol to methyl eugenol, the strong male attractant of *B. dorsalis* and other methyl eugenol-responding tephritid fruit fly species, we wanted to look both at *B. latifrons* response to methyl eugenol and *B. dorsalis* response to eugenol. We compared the attractiveness of 0.5 ml of eugenol + 1.0 ml of α-ionol, 0.5 ml of methyl eugenol + 1.0 ml of α-ionol, 0.5 ml of cade oil + 1.0 ml of α-ionol, 1.0 ml of α-ionol alone, 0.5 ml of eugenol alone, 0.5 ml of methyl eugenol alone, and 0.5 ml of cade oil alone to sterile *B. latifrons* and wild *B. dorsalis.* Tests were run with 10 traps of each treatment set out in a randomized complete block design in a macadamia nut orchard, as described for study 1. To provide some aging of treatments, fresh sticky cards were placed in the Jackson traps 1 wk after initial deployment. Immediately thereafter, sexually mature sterile *B. latifrons* adults were released uniformly throughout the trapping grid. Although, macadamia nut is not a *B. dorsalis* host, there was an established *B. dorsalis* population in the area that responded to the traps in this study. Sticky inserts from the Jackson traps were recovered 24 h after the fly release, with counts made for both *B. latifrons* and for *B. dorsalis.*

Field Validation Test with Wild Flies. Attraction of wild male *B. latifrons* to α-ionol with eugenol was compared with attraction of α-ionol with cade oil by using paired traps set out at two sites on Maui, known to have established *B. latifrons* populations. Site A included part of Huluhulunui Gulch north of Kokomo, with traps also set within a smaller, adjacent gulch and on a ridge between the two gulches. Site B was downstream of this site in Haiku. These sites both had well developed thickets of turkey berry, *Solanum torvum* Sw., with site A also having some scattered plants of Sodom apple, *Solanum linnaeanum* Hepper & P. Jaeger, both known hosts of *B. latifrons* (Liquido et al. 1994). Two Jackson traps were set out at each of 12 subsites (site A) and 10 subsites (site B). Each trap had two small plastic baskets, each holding a 3.8-cm-long by 1.0-cm-diameter cotton wick, hung from the trap hangers. One wick in each trap held 2.0 ml of α-ionol, whereas the second wick held either 1.0 ml of cade oil (cade oil trap) or 1.0 ml of eugenol (eugenol trap). At each subsite, one eugenol trap and one cade oil trap were hung in turkey berry plants, separated by $\approx$10 m, with the order of placement of the pair randomized. Traps were set out at both sites on 19 November 2002. At both sites, traps were serviced weekly

for four consecutive weeks, with trap positions reversed at each subsite at weeks 1, 2, and 3.

Comparative Test of Distance of Response. Catch at traps baited with eugenol + α-ionol were relatively much less than those baited with cade oil + α-ionol in the wild fly test compared with the results in study 2 with sterile flies and in other sterile fly tests using relatively low α-ionol: eugenol ratios (see Results). One theory that could explain why performance with wild flies was noticeably worse than with sterile flies was that sterile flies were released rather close to the traps whereas, in the field, the α-ionol + cade oil traps might have a better draw at greater distances and thus be able to draw in flies from a greater distance. To test this theory, flies were successively (on the same morning) released at three different distances (5, 13, and 23 m) from a row of Jackson traps alternately baited with either 2.0 ml of α-ionol + 1.0 ml of cade oil or 2.0 ml of α-ionol + 1.0 ml of eugenol. Ten traps of each treatment were alternated down a row of macadamia nut trees, with sterile flies released on either side of that row (5 m away), in the alleys beyond the next rows of trees (13 m) and in the alleys separated by two rows of macadamia nut trees (23 m). Sticky inserts were recovered 24 h after the fly release. After the initial test, all traps were left to age and releases, followed by 24-h insert recoveries, were repeated 1 and 2 weeks after the initial trap deployment.

Statistical Analyses. For studies 1–5 and the comparative test of distance of response (all tests using sterile laboratory flies), all trap catch results were square root transformed [sq rt (x + 0.5)] before analysis. The difference in catch among treatments was tested using an analysis of variance (ANOVA) on the transformed values followed by either a Waller–Duncan K-ratio T test (studies 1–4) (SAS Institute 1998) or the Bonferroni pairwise procedure (study 5) (SPSS Science 2000) for separation of means. For the comparative test of distance of response, difference in catch between treatments was tested using *t*-tests on square-root transformed data. For studies 1–5 and the comparative test of distance of response, untransformed trap catch results are presented together with statistical results based on transformed values. For the field validation test with wild flies, difference in trap catch between treatments was tested with paired *t*-tests of square-root transformed data for each week at each site (SPSS Science 2000).

Results

Field Tests with Sterile Laboratory Flies. *Study 1. Initial Field Trials.* Average male catch in the two different ratio trials are presented in Fig. 2. In both tests, there were significant differences in male catch among treatments (study A: $F = 62.42$, df = 9,140; $P < 0.0001$; and study B: $F = 29.54$, df = 9,140; $P < 0.0001$). In both tests, there were three general groupings: cade oil had the best synergistic response; followed by eugenol, isoeugenol, 2-methoxy-4-ethylphenol, and 2-methoxy-4-propylphenol; and finally, crotonaldehyde, *o*-eugenol, isovanillin, and α-ionol alone. The latter group showed very little synergistic effect because catch in these treatments was typically not significantly different from catch with α-ionol alone. Catch with compounds in the second group were all significantly greater than catch with compounds in the third group, but they were significantly less than with cade oil, except for eugenol at the 1:50 ratio. Typically, catch in the second group improved relative to cade oil as the relative concentration of the test compound increased. This was also true when comparing catches in the 1:1 set with the 1:4 set (unpublished data).

Study 2. Trials with Pure Compounds. Average male catch in this test is presented in Fig. 3. There was a significant difference in male catch among treatments ($F = 42.38$, df = 9,140; $P < 0.0001$). Although average catch at cade oil treated traps was numerically greater than at other treatment traps, it was not statistically greater than at traps treated with eugenol, 2-methoxy-4-methylphenol, or 2-methoxy-4-propylphenol. Catch at traps treated with 2-methoxy-4-ethylphenol, as well as catch at traps treated with the two mixtures, was significantly less than at the cade oil-treated traps, but not significantly less than catch at traps treated with eugenol, 2-methoxy-4-methylphenol, or 2-methoxy-4-propylphenol. Catch at traps treated with isoeugenol was not significantly different from catch at traps treated with 2-methoxy-4-ethylphenol or the two combinations but was significantly greater than catch at traps treated with α-ionol alone.

Study 3. Weathering Test with Eugenol. Average trap catch was significantly different ($P < 0.001$, df = 10, 44) each week of the test. *F* values were 16.77, 30.38, 26.90, 19.16, 9.99, and 28.97 for weeks 0, 1, 2, 4, 6, and 8, respectively. Average male trap catch for each treatment at each week is presented in Table 1, together with results of the mean separations. At week 0, there was no significant difference in catch between the cade oil + α-ionol treatments and the aged 1.0 ml of eugenol + α-ionol treatment, although the fresh 1.0 ml of eugenol + α-ionol treatment (which at week 0 was identical to the fresh treatment) had significantly lower catch. At weeks 1, 2, and 4, the aged 1.0 ml of eugenol + α-ionol treatment had the highest average catch of any treatment, with catch significantly greater than catch in any other treatment at weeks 1 and 4. At week 6, average catch at the aged 1.0 ml of eugenol + α-ionol treatment was less than, but not significantly different from, both the fresh cade oil + α-ionol treatment and the fresh 1.0 ml of eugenol + α-ionol treatment. At week 8, fresh, but not aged, 1.0 ml of eugenol + α-ionol treatments had significantly less catch than at the fresh cade oil + α-ionol treatment. Catch at traps with the higher eugenol loading was typically higher than at the traps with lower eugenol loading of comparable aging status. Average catch at traps baited with eugenol alone was always significantly less than catch at traps baited with α-ionol alone, irrespective of weathering status.

Study 4. Test of Mixing Effect. Average trap catch was significantly different ($P < 0.001$; df = 10, 44) each week of the test. *F* values were 23.85, 9.93, 20.55, and

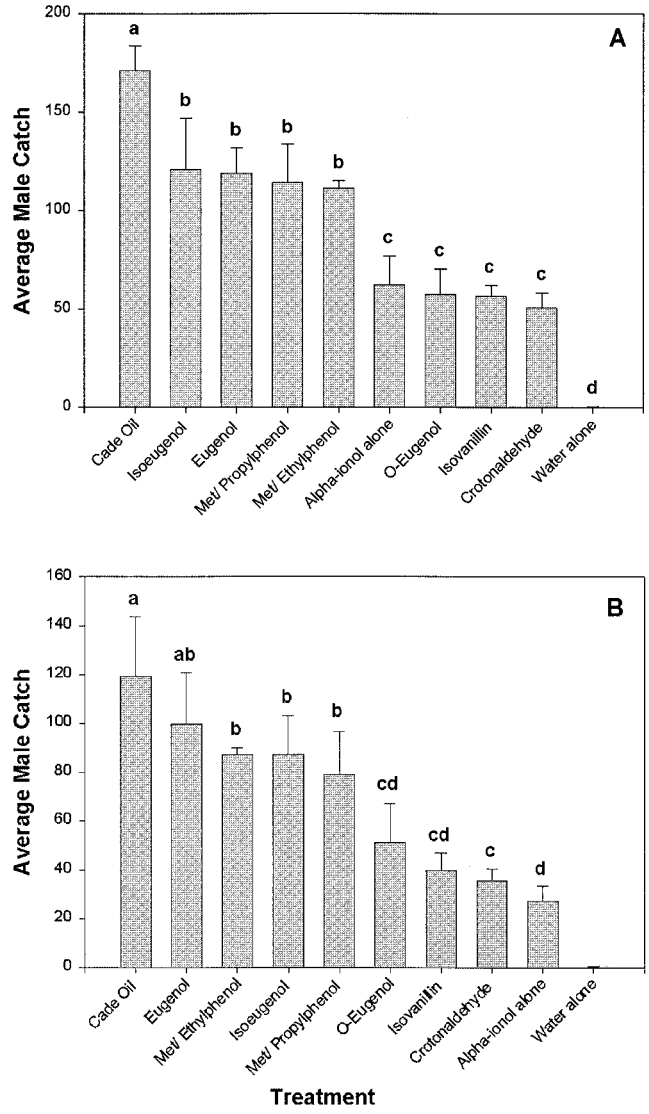


Fig. 2. Average sterile male *B. latifrons* catch at traps baited with 0.4 ml of α-ionol and 0.1 ml of cade oil, 0.4 ml of α-ionol alone, 0.4 ml of water alone, or with (A) 0.4 ml of a 5,000 ppm methanol solution of each of seven potential synergists identified in cade oil each presented with 0.4 ml of α-ionol, producing a ratio of 1 part potential synergist to 200 parts α-ionol; or with (B) 1.6 ml of a 5,000 ppm methanol solution of each of seven potential synergists identified in cade oil each presented with 0.4 ml of α-ionol, producing a ratio of 1 part potential synergist to 50 parts α-ionol. Means with the same letter are not significantly different (at the $\alpha = 0.05$ level) based on ANOVA of square-root transformed trap catch data.

10.31 for weeks 0, 2, 4, and 6, respectively. Average male trap catch for each treatment at each week is presented in Table 2, together with results of the mean separations. The average catch at aged unmixed cade oil traps was greater than at aged mixed cade oil traps at weeks 0, 2, 4, and 8, with catch significantly greater at weeks 2 and 6. Similarly, the average catch at aged unmixed eugenol traps was greater than at aged mixed eugenol traps at weeks 0, 2, 4, and 8, with catch significantly greater at weeks 0, 2, and 4. Differences in mixed versus unmixed catch with fresh cade oil and α-ionol wicks were not significant at any week. However, average catch at traps with unmixed fresh eugenol and α-ionol wicks exceeded that at traps with mixed fresh eugenol and α-ionol wicks at weeks 0, 2, 4, and 6, with catch significantly greater at weeks 0 and 6. In unmixed aged traps, trap catch at cade oil traps was significantly greater than at eugenol traps at weeks 0 and 6, significantly less at week 4, and not significantly different at week 2. In mixed aged traps, average catch at cade oil-containing traps was significantly higher than at eugenol-containing traps at week 0, significantly less at week 6, and not significantly different at weeks 2 and 4.

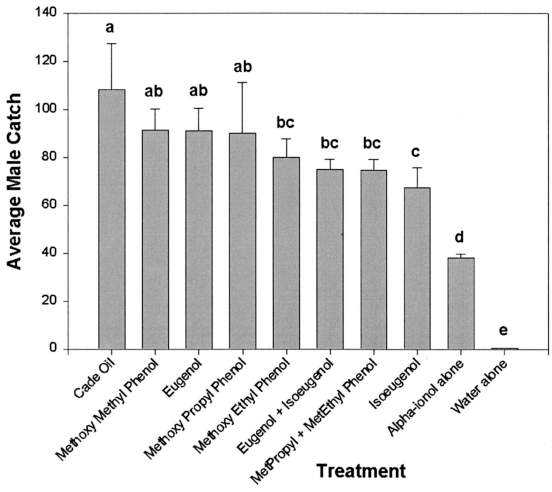


Fig. 3. Average sterile male *B. latifrons* catch at traps baited with 1.0 ml of water alone, 1.0 ml of α-ionol alone, 1.0 ml of α-ionol and 0.1 ml of cade oil, 1.0 ml of α-ionol and 0.1 ml of pure compounds of each of four potential synergists identified in cade oil, 1.0 ml of α-ionol and 0.1 ml of 2-methoxy-4-methylphenol, or 1.0 ml of α-ionol and 0.05 ml of each of two potential synergists, producing a ratio of 1 part potential synergist to 10 parts α-ionol. Means with the same letter are not significantly different (at the $\alpha = 0.05$ level) based on ANOVA of square-root transformed trap catch data.

Study 5. Documentation of Synergistic Effect and Oriental Fruit Fly Response. Average male *B. latifrons* trap catch results are presented in Fig. 4A and average male *B. dorsalis* trap catch results are presented in Fig. 4B. Average *B. latifrons* trap catch was significantly different among treatments ($F = 58.24$, df = 6, 63; $P < 0.0001$). Methyl eugenol alone (3.8 ± 1.1), eugenol alone (3.8 ± 1.0), and cade oil alone (0.7 ± 0.4) all showed low average catches. Although the addition of either eugenol or cade oil to α-ionol significantly increased the catch, the addition of methyl eugenol did not increase the catch significantly. A repeat ANOVA replacing the catches at traps baited with eugenol alone and with cade oil alone with the sum of the catch at traps baited with α-ionol alone and the catches of traps baited with either eugenol alone or cade oil alone also showed that there was a significant difference among treatments ($F = 10.28$, df = 6, 63; $P < 0.0001$), with the combination sums all significantly less than the catch at either α-ionol + cade oil or at α-ionol + eugenol. This shows that synergism is involved in the catch improvement both when eugenol is added to α-ionol as well as when cade oil is added to α-ionol.

Average *B. dorsalis* trap catch was significantly different among treatments ($F = 110.26$, df = 6, 63; $P < 0.0001$). There was catch at the eugenol only trap, but it was only approximately one-fifth of that at the methyl eugenol only trap. Traps baited with cade oil only, α-ionol only, cade oil + α-ionol, or eugenol + α-ionol failed to catch even one fly.

Field Validation Test with Wild Flies. Average male trap catch for cade oil traps and eugenol traps at each site at 1, 2, 3, and 4 wk is presented in Fig. 5. The average catch each week at each site was lower for the eugenol traps than for the cade oil traps, with the catch significantly less at weeks 1, 2, and 3 (Haiku) and weeks 2, 3, and 4 (Huluhulunui Gulch).

Comparative Test of Distance of Response. Average male trap catch results are presented in Table 3. There was no significant difference in male catch between treatments at any distance for any week in this test. The data, thus, provide no support that there may be a difference in distance of response to the two different attractant combinations.

Discussion

Four of seven compounds identified as potential active ingredients in cade oil (eugenol, isoeugenol, 2-methoxy-4-ethylphenol, and 2-methoxy-4-propylphenol) were found capable of synergizing the attractiveness of α-ionol to male *B. latifrons.* Eugenol showed only low direct attraction, but synergistically enhanced the attractiveness of α-ionol. The other three compounds similarly had low direct attraction (data not shown), but synergistically enhanced the attractiveness of α-ionol. The similar structure of these four identified synergistic compounds, together with a similar synergistic effect of a closely related com-

Table 1. Average male *B. latifrons* catch (±SEM) at 0, 1, 2, 4, 6, and 8 wk in traps baited with 2.0 ml of α-ionol and 0.2 ml of cade oil (cade), 2.0 ml of α-ionol, and either 0.2 ml or 1.0 ml of eugenol (eug), 2.0 ml of α-ionol alone, 1.0 ml of eugenol alone, or 2.0 ml of water alone

Treatment	Presentation	Wk 0	Wk 1	Wk 2	Wk 4	Wk 6	Wk 8
α-Ionol + 0.2 ml cade	Aged	41.8 ± 10.00a	17.0 ± 3.16cd	60.8 ± 9.09bc	24.2 ± 4.21cd	32.2 ± 7.03bc	33.6 ± 5.20cd
α-Ionol + 0.2 ml cade	Fresh	40.6 ± 9.91ab	35.4 ± 5.64b	86.0 ± 13.26ab	44.4 ± 13.43b	98.2 ± 26.02a	82.6 ± 11.83a
α-Ionol + 1.0 ml eug	Aged	35.0 ± 8.05abc	64.2 ± 15.25a	116.4 ± 28.28a	65.4 ± 9.62a	73.4 ± 41.92abc	27.0 ± 7.22d
α-Ionol + 1.0 ml eug	Fresh	20.2 ± 3.17cd	20.6 ± 2.01c	86.8 ± 13.14ab	33.6 ± 9.31bc	87.2 ± 15.37a	45.6 ± 2.09bc
α-Ionol + 0.2 ml eug	Aged	25.4 ± 6.67bcd	18.4 ± 2.29c	50.6 ± 9.11c	17.2 ± 2.56d	35.8 ± 4.09bc	26.4 ± 5.02d
α-Ionol + 0.2 ml eug	Fresh	17.0 ± 3.91d	13.4 ± 2.48cd	44.4 ± 7.60c	20.6 ± 3.76cd	66.0 ± 14.77ab	53.6 ± 11.29b
α-Ionol	Aged	17.6 ± 4.40d	5.6 ± 1.29e	22.4 ± 3.12d	6.8 ± 1.16e	27.2 ± 9.10c	27.8 ± 4.49d
α-Ionol	Fresh	16.4 ± 3.08d	9.2 ± 1.71de	41.8 ± 10.66cd	19.0 ± 2.47cd	32.0 ± 7.56bc	32.4 ± 8.35cd
1.0 ml eug	Aged	0.2 ± 0.20e	1.0 ± 0.55f	3.8 ± 1.11e	0.4 ± 0.24f	0.4 ± 0.40d	0.4 ± 0.40e
1.0 ml eug	Fresh	0.4 ± 0.24e	0.0 ± 0.00f	1.2 ± 0.58e	3.2 ± 2.06ef	4.6 ± 0.87d	1.4 ± 0.40e
2.0 ml water	Aged	0.0 ± 0.00e	0.0 ± 0.00f	0.0 ± 0.00e	0.0 ± 0.00f	0.0 ± 0.00d	0.0 ± 0.00e

Catch at both freshly baited traps and traps where the attractant(s) have been permitted to weather over time are presented for all treatments except the water treatment. For a given week, catches followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Table 2. Average male *B. latifrons* catch (±SEM) at 0, 2, 4, and 6 wk in traps baited with 2.0 ml of α-ionol and 1.0 ml of cade oil (cade) (mixed and on separate wicks), 2.0 ml of α-ionol and 1.0 ml of eugenol (eug) (mixed and on separate wicks), 2.0 ml of α-ionol alone, or 2.0 ml of water alone

Treatment	Mixed	Presentation	Wk 0	Wk 2	Wk 4	Wk 6
α-Ionol + 1.0 ml cade	Unmixed	Aged	290.2 ± 45.39a	64.0 ± 14.36a	41.8 ± 7.77cd	95.4 ± 24.00a
α-Ionol + 1.0 ml cade	Unmixed	Fresh	225.6 ± 38.31ab	43.6 ± 2.20abcd	56.0 ± 5.52bcd	61.0 ± 14.25ab
α-Ionol + 1.0 ml cade	Mixed	Aged	222.0 ± 34.06ab	33.6 ± 6.02cde	36.4 ± 7.67d	15.2 ± 6.70de
α-Ionol + 1.0 ml cade	Mixed	Fresh	156.8 ± 7.94bc	29.6 ± 6.50cdef	74.6 ± 9.15b	33.0 ± 5.46bcd
α-Ionol + 1.0 ml eug	Unmixed	Aged	135.2 ± 20.61c	59.6 ± 12.60ab	117.4 ± 27.30a	50.2 ± 21.07bc
α-Ionol + 1.0 ml eug	Unmixed	Fresh	155.4 ± 23.46c	52.4 ± 16.91abc	59.8 ± 8.01bc	93.8 ± 18.12a
α-Ionol + 1.0 ml eug	Mixed	Aged	70.8 ± 9.38d	25.2 ± 5.70def	45.6 ± 8.81cd	40.2 ± 9.04bc
α-Ionol + 1.0 ml eug	Mixed	Fresh	77.8 ± 18.91d	37.4 ± 9.68bcde	53.4 ± 8.00bcd	45.0 ± 7.58bc
α-Ionol	Unmixed	Aged	78.0 ± 16.36d	13.6 ± 3.06f	11.4 ± 1.75e	10.4 ± 2.66e
α-Ionol	Unmixed	Fresh	59.0 ± 8.62d	20.4 ± 2.01ef	12.4 ± 2.23e	25.0 ± 2.39cde
2.0 ml water	Unmixed	Aged	00.0 ± 0.00e	00.0 ± 0.00g	00.2 ± 0.20f	00.2 ± 0.20f

Catch at both freshly baited traps and traps where the attractant(s) have been permitted to weather over time are presented for all treatments except the water treatment. For a given week, catches followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

pound not found in cade oil (2-methoxy-4-methyl phenol) shows that there is a response to a basic 2-methoxy phenol structure, with fly response little affected by some variation in the composition of the side chain on the number 4 carbon. Of the five identified synergistic compounds, isoeugenol had previously been tested as a *B. latifrons* attractant, but, because attraction to it was much lower than to α-ionone (which showed attraction comparable to α-ionol), it was not considered further (Flath et al. 1994), nor was it tested as a synergist for α-ionol.

For both cade oil and eugenol synergistic enhancement of attraction of α-ionol to male *B. latifrons* is decreased if the synergist and α-ionol are mixed. For cade oil, this effect was found, generally, with comparisons of aged wicks but not with fresh wick comparisons. For eugenol, the difference was generally found for both fresh and aged wicks. This information should be used in setting up traps so as to maximize the catch response. It is for this reason that we place cade oil and α-ionol on separate wicks in Jackson traps that we use to detect/monitor *B. latifrons* populations.

Tests with sterile flies had been suggestive that α-ionol + eugenol may provide as good attraction to male *B. latifrons* as α-ionol + cade oil, with comparable trap catches even through 8 wk of aging. However, the tests with wild fly populations failed to confirm this. In McQuate and Peck (2001), field tests with wild flies confirmed results with sterile flies that showed that α-ionol + cade oil had improved attractiveness over α-ionol alone. Our test of one theory for this difference, that there may be a difference in the distance of response to the two attractant combinations, failed to show a significant difference. The result with the wild flies does not encourage using α-ionol + eugenol as an alternative to α-ionol + cade oil for routine detection and monitoring of *B. latifrons* populations.

One potential complication in assessing the effectiveness of potential synergists is that purchased test compounds, although highly purified, are not 100% pure. As an example, the lot of eugenol used in our

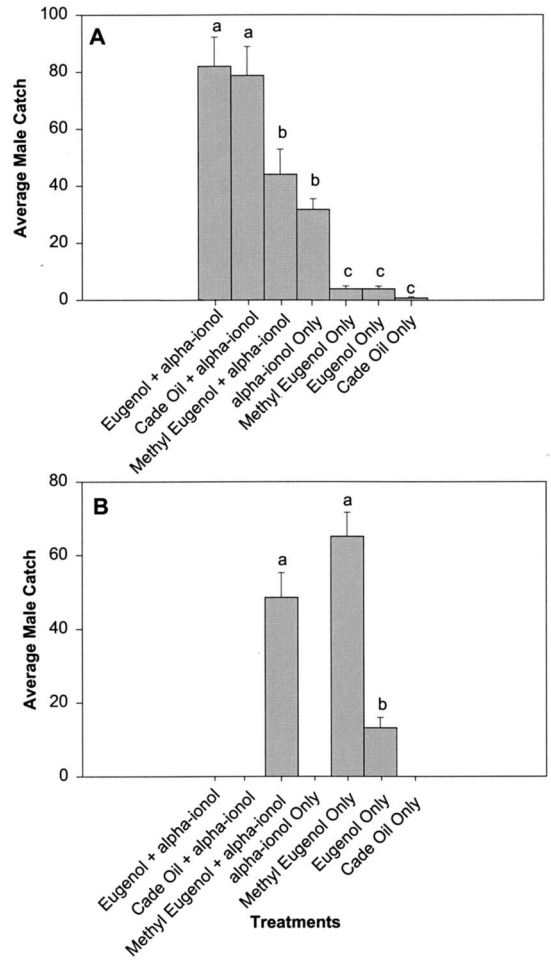
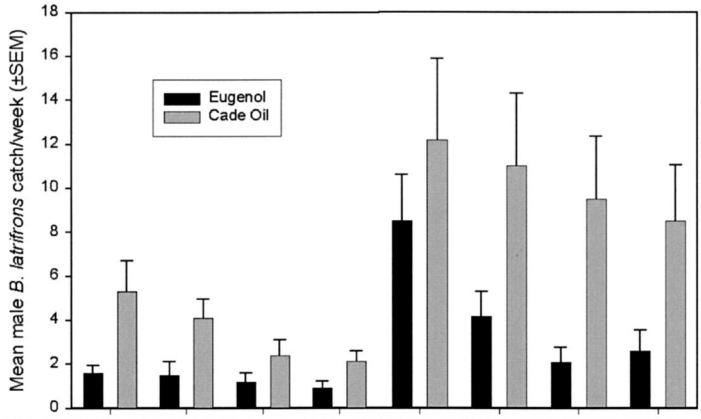


Fig. 4. Average catch of (A) sterile male *B. latifrons* and (B) wild male *B. dorsalis* at traps baited with α-ionol alone or α-ionol with or without eugenol, cade oil, or methyl eugenol. Means with the same letter are not significantly different (at the $\alpha = 0.05$ level) based on ANOVA of square-root transformed trap catch data.



	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
t	-3.381	-3.200	-2.301	-1.909	-1.151	-4.063	-3.512	-4.007
df	9	9	9	9	11	11	11	11
P	0.008*	0.011*	0.005*	0.089	0.274	0.002*	0.005*	0.002*
	Haiku				Huluhulunui Gulch			

Fig. 5. Average wild male *B. latifrons* catch at traps baited with 2.0 ml of α-ionol and 1.0 ml of cade oil or with 2.0 ml of α-ionol and 1.0 ml of eugenol at each of two sites at weeks 1, 2, 3, and 4. Also indicated are results of paired *t*-tests performed on square-root transformed trap catches for each week at each site.

tests was documented as being 99% pure. Therefore, in tests using 1.0 ml of eugenol, 0.01 ml of impurity would be included. This quantity is more than the quantities of individual compounds tested in our trial 1, and we found synergistic effect at the concentrations tested there. Impurities in our test compounds, although in very low concentrations, could have an effect on fly response, either positively or negatively.

It could be that the synergistic effect of cade oil may result from an additive effect of several compounds. Several combinations were tested in this study and not found to have any enhanced synergistic effectiveness, but because all possible combinations were not tested, the possibility cannot be ruled out that there may be certain combinations that could produce an enhanced synergistic effect. Such an enhanced synergistic effect, resulting from combinations of ingredients, may be more of a combined dose effect because of the structural similarity of the identified synergists. Enhanced synergistic effect was found as the concentration of individual identified compounds increased, so if similar compounds have essentially the same effect, their combined presence could provide an increased synergist concentration. However, it could also be possible that another component from cade oil of dissimilar structure may be involved that could stimulate another receptor type on the antennae. Of the potential synergists tested here, only crotonaldehyde differed significantly in structure. We, however, did not conduct any combination tests with this compound in the course of this study.

One interesting note with the identified compounds is that none of them have the characteristic "smoky/barbecue" odor of cade oil. This pronounced odor, so noticeable to the human sense of smell, is apparently not a factor in the response of *B. latifrons.* This indicates that there is a potential to develop the male attractant for *B. latifrons* so that it lacks the strong, penetrating odor, which would be an improvement for those responsible for deploying detection/monitoring traps for this species.

The similarity of the identified synergists in cade oil to methyl eugenol, the most powerful of the tephritid fruit fly male lures (Cunningham 1989), is intriguing.

Table 3. Average catch (±SEM) of sterile male *B. latifrons* 24 h after fly release at traps baited with either α-ionol + cade oil or α-ionol + eugenol when flies were released at three different distances away from the traps, and repeated on three successive weeks without recharging the bait

	5 m from traps		13 m from traps		23 m from traps	
	α-ionol + cade	α-ionol + eug	α-ionol + cade	α-ionol + eug	α-ionol + cade	α-ionol + eug
Wk 0	3.47 ± 0.39	4.06 ± 0.38	2.23 ± 0.27	2.80 ± 0.25	1.48 ± 0.21	1.81 ± 0.30
Wk 1	4.03 ± 0.33	4.80 ± 0.33	2.93 ± 0.44	3.19 ± 0.24	3.20 ± 0.42	3.02 ± 0.29
Wk 2	4.28 ± 0.34	5.54 ± 0.49	1.50 ± 0.20	1.83 ± 0.25	2.52 ± 0.24	1.99 ± 0.31

Differences in catch between treatments were not significant for any distance in any week.

B. latifrons has been one of those tephritid fruit fly species of the subfamily Dacinae that failed to fit into the dichotomy of methyl eugenol-responding versus cuelure-responding species (Drew 1974, Drew and Hooper 1981, Metcalf 1990), yet this research has shown synergism of the established lure (α-ionol) with a compound (eugenol) very closely related to methyl eugenol. As shown in this study, *B. latifrons* does show a very low level direct response to both eugenol and to methyl eugenol, but the methyl eugenol does not enhance the attractiveness of α-ionol to male *B. latifrons.* However, we have also shown that *B. dorsalis* shows a low level response to eugenol but not to cade oil. *Bactrocera latifrons* and *B. dorsalis* are both in the *Bactrocera* subgenus of the genus *Bactrocera*, although, beyond that, it is not clear how closely related these two species are. Even in the subgenus *Bactrocera*, there are methyl eugenol-responding and cue lure-responding species as well as species, such as *B. latifrons*, not directly responding to either lure (White and Elson-Harris 1992, Drew and Hancock 2000).

The identification of active components of cade oil opens additional paths of research to further develop a male lure for *B. latifrons.* One possible approach would be to systematically assess the synergistic effectiveness of structural variants of the basic 2-methoxy phenol structure as was done for attractiveness of structural variants of methyl eugenol to *B. dorsalis* (DeMilo et al. 1994). Another approach could be to seek to develop a single compound that incorporates the effects of both the attractant and the synergist. We prefer to better understand the biology behind the observed synergism through electroantennogram recordings of action potentials generated by both concurrent and sequential antennal exposure to α-ionol and identified synergists. (Dickens and Payne 1978).

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